Fungi from decayed wood as ectomycorrhizal symbionts of western hemlock[1,2]

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Four wood-inhabiting fungi, *Byssoporia terrestris*, *Cenococcum geophilum*, *Piloderma bicolor*, and an unidentified isolate, formed mycorrhizae with western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in pure culture. Field observations indicate that *Cantharellus infundibuliformis*, *Chroogomphus tomentosus*, *Galerina* sp., and *Russula* sp. are also mycorrhizal with hemlock growing in rotten wood.

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Quatre champignons lignicoles *Byssoporia terrestris*, *Cenococcum geophilum*, *Piloderma bicolor* et un isolant non-identifié ont formé en culture pure des mycorhizes sur la pruche de l'Ouest (*Tsuga heterophylla* (Raf.) Sarg.). Des observations sur le terrain montrent que *Cantharellus infundibuliformis*, *Chroogomphus tomentosus*, *Galerina* sp. et *Russula* sp. sont aussi capables de former des mycorhizes avec la pruche croissant sur du bois pourri.

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Introduction

Decayed wood is an important substrate for ectomycorrhizal activity. Harvey *et al.* (1976) have shown that rotten wood in Douglas-fir–larch stands in Montana supports a substantial portion of the total number of ectomycorrhizae in the forest floor. They found that rotten wood supports more active mycorrhizae during the drier period of the year than soil humus (Harvey *et al.* 1978). They have also determined that, although rotten wood supports more of the active mycorrhizae on a dry site than a wet one, it is an important substrate for mycorrhizal associations of moist hemlock habitat types in the northern Rockies (Harvey *et al.* 1979). Western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) commonly regenerates on decaying logs and stumps in old-growth forests in the Pacific Northwest (Minore 1972). Most natural hemlock seedlings on decayed wood are mycorrhizal. Nonmycorrhizal seedlings outplanted in rotten wood in clear-cuts become mycorrhizal during the first 2–3 months after planting (B. R. Kropp, unpublished data). Rotten wood from a clear-cut and from a forest has also been used successfully to inoculate containerized hemlock seedlings with mycorrhizal fungi (B. R. Kropp, unpublished data).

Although many fungi have been identified as being mycorrhizal with hemlock (Trappe 1962; B. R. Kropp and J. M. Trappe, unpublished data; Zak 1969; R. Molina, unpublished data), almost no attempt has been made to identify fungi that form mycorrhizae with hemlock in rotten wood. Trappe (1965) reported that tuberculate mycorrhizae occurring on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) are found most abundantly in well-rotted stumps and logs. The fungus forming this mycorrhiza was later identified as *Rhizopogon vinicolor* A. H. Smith (Zak 1971*b*). Zak (1976) has found the wood-inhabiting fungus *Piloderma* (*Corticium*) *bicolor* to be mycorrhizal with Douglas-fir and Pacific madrone (*Arbutus menziesii* Pursh). Another wood-inhabiting fungus, *Piloderma byssinum* (Karst.) Jülich, is mycorrhizal on Douglas-fir (Froidevaux 1975). *Byssoporia terrestris* has been shown to be mycorrhizal in rotten wood with both Douglas-fir and western hemlock (Zak 1969; Larsen and Zak 1978). This is the only report of an identified wood-inhabiting fungus mycorrhizal with western hemlock.

The objective of this research was to identify additional wood-inhabiting fungi which form mycorrhizae with western hemlock.

Materials and methods

Field observations and pure culture syntheses were used to determine whether fungi were mycorrhizal with western hemlock. Sporocarps occurring on rotten wood near hemlock

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seedlings were collected from sites in western Oregon and identified. If the sporocarps were found within about 50 cm of a hemlock seedling on rotten wood, the fungi were considered as possible symbionts with the seedlings. Although no definite rhizomorph or mycelial connections were found between the sporocarps and seedlings, some of the seedlings had mycorrhizae that were covered with mycelium that visually resembled that at the base of the sporocarps. Where mycelium at the base of the sporocarp and on the mycorrhizae appeared to be the same, field indications of a mycorrhizal association were considered positive. However, visual observations of this type are subjective and the association was not considered conclusive unless it could be obtained in pure culture synthesis. Most of the pure cultures of fungi were obtained by tissue explant from sporocarps. *Cenococcum geophilum* was obtained from surface-sterilized sclerotia (Trappe 1969). Isolate B and *Piloderma bicolor* (Table 1) were obtained from hemlock mycorrhizae in rotten wood (Zak and Marx 1964). Isolate C and *Byssoporia terrestris* were cultured from surface-sterilized rhizomorphs. Cultures of *Fomitopsis officinalis* and *Perenniporia subacida*, which are commonly found in decaying logs, were obtained from the Forest Products Laboratory at Madison, Wisconsin (Table 1).

Each fungus obtained in culture was tested in pure culture synthesis tubes containing a peat–vermiculite mix and a nutrient solution, following the methods used by Molina (1979*a*). Hemlock seeds were rinsed in running tap water for 1 h and shaken in Tween 20 for 30 min. They were again rinsed in running water for 1 h before being surface sterilized by shaking in 30% H_2O_2 for 30 min. The seeds were then rinsed aseptically with 2 L of sterile distilled H_2O and placed on agar in vials. After 1 week incubation at room temperature and disposal of contaminated vials, sterile seeds were stratified in the vials for 3–4 weeks at 3–4°C. They were then germinated under fluorescent–incandescent light at room temperature, planted aseptically into pure culture synthesis tubes, and inoculated with a mycelial slurry of the desired fungus. The tubes in which seedlings were grown were half submerged in a 16°C water bath under fluorescent–incandescent lighting (10 500 lx) with a 15-h photoperiod. After 4–5 months, they were examined for mycorrhiza formation. The presence of a Hartig net on roots squash mounted in 5% KOH was considered evidence of mycorrhiza formation.

Results and observations

Fourteen fungi (Table 1) were found on rotten wood in which western hemlock seedlings were growing. Nine of these were tested with hemlock in pure culture synthesis; the remaining five were not obtained in culture. Cultures of four additional wood-inhabiting fungi were also tested in pure culture synthesis with western hemlock. Pure culture synthesis showed that four fungi form mycorrhizae with hemlock. Field observations strongly indicated that four additional fungi (*Cantharellus infundibuliformis*, *Chroogomphus tomentosus*, *Galerina* sp., and *Russula* sp.) form mycorrhizae with hemlock growing in wood.

TABLE. 1. Fungi occurring in rotten wood and results of attempted pure culture syntheses of mycorrhizae with *Tsuga heterophylla*

Species	Field observations*	Pure culture synthesis†
Boletus mirabilis Murrill	*a*	−,−
Byssoporia terrestris (D. C. per Fries) Larsen et Zak	*b*	+
Cantharellus infundibuliformis Fr.	*b*	No culture
Cenococcum geophilum Fr.	*b*	+
Chroogomphus tomentosus (Murr.) O. K. Miller	*a*	No culture
Heterobasidion annosum (Fr.) Bref.	*c*	−
Fomitopsis officinalis (Vill. ex Fr.) Bond et Sing.	*c*	−
Galerina sp.	*b*	No culture
Isolate B	*b*	+
Isolate C	*a*	−
Lactarius rufus var. *parvus* Hesler et Smith	*a*	−
Paxillus atrotomentosus (Batsch ex Fr.) Fries	*b*	−,−
Perenniporia subacida (Peck) Donk	*c*	−
Piloderma bicolor (Peck) Jülich	*b*	+
Pleurotus porrigens (Fr.) Gillet	*a*	No culture
Russula sp.	*b*	No culture
Tricholomopsis rutilans (Fr.) Sing.	*c*	−
Xeromphalina brunneola O. K. Miller	*b*	−

**a*, observed with hemlock; *b*, observed with hemlock as an apparent mycorrhizal associate; *c*, inhabits wood, not observed with hemlock.

†Synthesis attempted twice with certain fungi; + = ectomycorrhizae formed, − = no ectomycorrhizae formed.

Cenococcum geophilum, a common forest soil fungus that is also found in decayed wood, formed mycorrhizae with hemlock in pure culture. This fungus was also observed on natural hemlock seedlings growing in wood. *Cenococcum* was the predominant fungus which formed mycorrhizae on nonmycorrhizal seedlings planted in rotten wood in clear-cuts and on containerized seedlings inoculated with rotten wood (B. R. Kropp, unpublished data). *Cenococcum* has also been reported by Trappe (1962) to form mycorrhizae with western hemlock in the field.

Piloderma bicolor is known to be mycorrhizal with Douglas-fir in nature and in pure culture (Zak 1976, 1971*a*). The results presented in Table 1 show that it also forms mycorrhizae with hemlock in pure culture synthesis and in nature. In addition, inoculations of containerized western hemlock with rotten wood containing this fungus resulted in *Piloderma* mycorrhizae (B. R. Kropp, unpublished data).

Zak (1969) and Larsen and Zak (1978) reported field observations of *Byssoporia terrestris* as being mycorrhizal with both western hemlock and Douglas-fir. Zak (1969) reported difficulty in obtaining mycorrhizae on Douglas-fir with *Byssoporia* in pure culture synthesis, although one attempt was successful. He made no attempt to test *Byssoporia* with hemlock in pure culture. The present results (Table 1) support his field observations of an association between hemlock and *Byssoporia* by demonstrating that *Byssoporia* mycorrhizae do form in pure culture synthesis.

In the field, isolate B had heavily colonized the seedling from which it was obtained. It also formed mycorrhizae in pure culture synthesis. However, mycorrhiza formation was poor, with only one mycorrhiza developing on one of the seedlings. No sporocarps were available for identification.

The culture of *Lactarius rufus* var. *parvus*, from rotten wood, failed to form mycorrhizae with hemlock in pure culture. A successful synthesis, however, was made with a culture of *L. rufus* var. *rufus* obtained from R. Molina. This variety is commonly found fruiting on humus while *Lactarius rufus* var. *parvus* is found on decaying wood (Hesler and Smith 1979).

Attempts to synthesize mycorrhizae in pure culture with two isolates of *Boletus mirabilis* and one of *Paxillus atrotomentosus* gave negative results. However, the mycelium forming the hemlock mycorrhizae found with the collection of *P. atrotomentosus* was superficially identical in color and texture to that of the sporocarp base. This, plus the field association between the sporocarps and mycorrhizae indicate that *Paxillus* is a likely mycorrhizal associate of western hemlock. *Boletus mirabilis*, on the other hand, appears less likely to be mycorrhizal with hemlock. Even though the collected sporocarp was found about 5 cm from several small hemlock seedlings, examination of hemlock mycorrhizae at the base of the sporocarp showed no mycorrhizae with mycelium resembling that of the sporocarp. Interestingly enough, some of the mycorrhizae found appressed to the sporocarp base were formed by *Cenococcum*.

Cantharellus infundibuliformis, *Russula* sp., and *Galerina* sp. were closely associated with hemlock on rotten wood but were not obtained in culture. The superficial similarities in color and texture between mycelium-forming mycorrhizae on hemlock seedlings and that at the base of the sporocarps indicate that these fungi are likely to be mycorrhizal with hemlock.

Chroogomphus tomentosus was observed with hemlock growing on wood, along with mycorrhizae formed with mycelium from the sporocarp. These mycorrhizae, however, could not be positively identified as belonging to western hemlock.

Two fungi, *Xeromphalina brunneola* and *Pleurotus porrigens*, both considered saprophytic, were found with hemlock seedlings on rotten logs. Mycorrhizae were found within the tissue of *Pleurotus* sporocarp. Since no culture grew from tissue explants from this fungus, it could not be tested in pure culture synthesis. Since *Pleurotus* is a saprophytic genus, it is unlikely to be mycorrhizal with hemlock.

Xeromphalina brunneola was also found with hemlock, along with mycorrhizae having similar mycelium. This fungus grew in culture but did not form mycorrhizae in pure culture synthesis.

Tricholomopsis rutilans from wood was cultured and tested with hemlock in pure culture with negative results; it was not observed with seedlings in the field and should be considered saprophytic.

Heterobasidion annosum was tested in pure culture synthesis with hemlock because it had been reported by Orlos and Dominik (1960) to form an unusual type of mycorrhiza in culture with pine seedlings. Hemlock seedlings were killed by this fungus.

Fomitopsis officinalis and *Perenniporia subacida* both failed to form mycorrhizae in pure culture. These fungi were selected because they were originally isolated from Oregon sources and because they are commonly isolated from decaying logs. It was thought that these fungi might be able to persist in downed logs and eventually become mycorrhizal with seedlings that became established on the logs.

Discussion

Some fungi that form mycorrhizae on hemlock seedlings growing in decayed wood on clear-cuts are the same as those that form mycorrhizae with hemlock in soil (B. R. Kropp, unpublished data). It is likely that, as logs and stumps decay and become incorporated into the soil, soil-inhabiting mycorrhizal fungi are able to colonize the rotten wood and form mycorrhizae with hosts growing in the wood. *Cenococcum geophilum* is an example of a mycorrhizal fungus which occurs in both soil and wood. Other fungi are restricted to rotten wood (B. R. Kropp, unpublished data); however, even some of these fungi are able to colonize soil as reported for *Byssoporia terrestris* by Zak (1969). The author has observed mycelia of *Piloderma bicolor* growing in soil near rotten wood. Another possibility is that different varieties or ecotypes of the same fungus may occupy rotten wood and soil as is the case with *Lactarius rufus* varieties *parvus* and *rufus*.

Numerous decaying logs in old-growth forests in the Pacific Northwest are suspended above the soil. These logs are often covered with mycorrhizal western hemlock seedlings. The fungi forming these mycorrhizae may have been living in the log saprophytically before it fell or may have colonized it afterwards. There are instances of fungi from saprobic genera which have been shown to be mycorrhizal with orchids (Furman and Trappe 1971).

In this research, two fungi commonly found in standing or recently felled trees failed to form mycorrhizae. This suggests that fungi which form mycorrhizae colonize the wood after the tree has fallen. However, much more research needs to be done to validate this suggestion.

Both of the cultures used were quite old. *Fomitopsis officinalis* had been isolated in 1960 and *Perenniporia subacida* had been isolated in 1971. Marx (1981) has found that the ability of *Pisolithus tinctorius* (Pers.) Coker and Couch to form mycorrhizae can decrease over long periods of time in culture. If the two fungi from rotten wood originally had the ability to form mycorrhizae, they may have lost their vigor in culture. However, fungi may retain the ability to form mycorrhizae over long periods of time in culture. An isolate of *Thelephora americanus* Lloyd, maintained in culture since 1965, formed mycorrhizae although no data are available to determine whether the vigor of mycorrhiza formation has decreased over time (B. R. Kropp and J. M. Trappe, unpublished data).

Because of the artificial conditions of pure culture synthesis, negative results do not necessarily mean that a fungus cannot form mycorrhizae with the host in nature. In this research, several fungi that were observed in association with hemlock on rotten wood did not form mycorrhizae in culture. These might be able to colonize hemlock under natural conditions. Isolate B formed abundant hemlock mycorrhizae in nature but only one mycorrhiza in culture.

Different isolates of the same fungal species may vary in their ability to form mycorrhizae. Molina (1979*b*) found that different isolates of *Pisolithus tinctorius* varied in their ability to colonize short roots of lodgepole pine (*Pinus contorta* Dougl.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). With the exceptions of *Lactarius rufus* and *Boletus mirabilis*, only one isolate of each of the fungi tested in this study was used. If more isolates of the fungi giving negative results are obtained, they may be shown to form mycorrhizae in culture.

Field observations of mycorrhizae on western hemlock seedlings indicate that many additional mycorrhizal fungi inhabit rotten wood. Further collections and pure culture syntheses are needed to identify them.

FURMAN, T. E., and J. M. TRAPPE. 1971. Phylogeny and ecology of mycotrophic achlorophyllous angiosperms. Q. Rev. Biol. **46**: 219–225.

FROIDEVAUX, L. 1975. Identification of some Douglas-fir mycorrhizae. Eur. J. For. Pathol. **5**: 212–216.

HARVEY, A. E., M. F. JURGENSEN, and M. J. LARSEN. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest in western Montana. For. Sci. **24**: 203–208.

HARVEY, A. E., M. J. LARSEN, and M. F. JURGENSEN. 1976. Distribution of ectomycorrhizae in mature Douglas-fir/larch forest soil in western Montana. For. Sci. **22**: 393–398.

——— 1979. Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat types. For. Sci. **25**: 350–358.

HESLER, L. R., and A. H. SMITH. 1979. North American species of *Lactarius*. The University of Michigan Press, Ann Arbor.

LARSEN, M. J., and B. ZAK. 1978. *Byssoporia* gen. nov.: taxonomy of the mycorrhizal fungus *Poria terrestris*. Can. J. Bot. **56**: 1122–1129.

MARX, D. H. 1981. Variability in ectomycorrhizal development and growth among isolates of *Pisolithus tinctorius* as affected by source, age, and reisolation. Can. J. For. Res. **11**: 168–174.

MINORE, D. 1972. Germination and early growth of coastal tree species on organic seed beds. U.S., For. Serv., Res. Pap. PNW-135.

MOLINA, R. 1979*a*. Pure culture synthesis and host specificity of red alder mycorrhizae. Can. J. Bot. **57**: 1223–1228.

——— 1979*b*. Ectomycorrhizal inoculation of containerized Douglas-fir and lodgepole pine seedlings with six isolates of *Pisolithus tinctorius*. For. Sci. **25**: 585–590.

ORLOS, H., and T. DOMINIK. 1960. Zbiologii huby korzeniowej—(*Fomes annosus* (Fr.) Cooke). Sylwan, **104**: 1–13.

TRAPPE, J. M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. **28**: 538–605.

——— 1965. Tuberculate mycorrhizae of Douglas-fir. For. Sci. **11**: 27–32.

——— 1969. Studies on *Cenococcum graniforme*. I. An efficient method for isolation from sclerotia. Can. J. Bot. **47**: 1389–1390.

ZAK, B. 1969. Characterization and classification of mycorrhizae of Douglas-fir. I. *Pseudotsuga menziesii* + *Poria terrestris* (blue- and orange-staining strains). Can. J. Bot. **47**: 1833–1840.

——— 1971*a*. Characterization and classification of mycorrhizae of Douglas-fir. II. *Pseudotsuga menziesii* + *Rhizopogon vinicolor*. Can. J. Bot. **49**: 1079–1084

——— 1971*b*. Detoxification of autoclaved soil by a mycorrhizal fungus. U.S., For. Serv., Res. Note PNW-159.

———1976. Pure culture synthesis of Pacific madrone ectendomycorrhizae. Mycologia, **68**: 362–369.

ZAK, B., and D. H. MARX. 1964. Isolation of mycorrhizal fungi from roots of individual slash pines. For. Sci. **10**: 214–222.